Effects of incubation temperature on hatchling phenotypes in an oviparous lizard with prolonged egg retention: are the two main hypotheses on the evolution of viviparity compatible?

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ABSTRACT

Females of several lizard species modify their body temperature during pregnancy, probably in connection with the optimisation of hatchling phenotypes. We studied variations in the temperature selected by gravid females compared with those selected by males and non-gravid females in an oviparous population of *Zootoca vivipara* (Jacquin, 1797) (Squamata: Lacertidae) of Northern Spain and examined the effects of incubation temperature on the phenotypic variation of hatchlings. Cloacal temperatures of gravid females active in the field were lower than those of males and non-gravid females, as well as the temperatures selected in a thermal gradient created in the laboratory (mean ± s.d.: 32.33 ± 1.27 °C for gravid females; 34.05 ± 1.07 °C for males and non-gravid females). Effects of temperature were assessed by incubating eggs at five constant temperatures (21, 25, 29, 32 and 34 °C). Incubation time decreased as temperature increased, following a negative exponential function. Incubation temperatures also affected the hatchlings' morphology: hatchlings incubated at 34 °C had shorter heads than those from other temperatures. Survival at 34 °C (58%) was significantly lower than at the other temperatures (mean 93%). Pregnant females select lower body temperature, approaching the temperatures that optimise hatchling phenotypes, according to predictions of the maternal manipulation hypothesis on the evolution of viviparity. The shift in preferred temperature by pregnant females would result in only a very short delay, if any, of hatching time and, because the temperature selected by pregnant females is much higher than average temperatures recorded in natural nests of *Z. vivipara*, egg retention considerably shortens incubation time, according to predictions of the cold-climate hypothesis. Our experimental results indicate that the two main hypotheses on the evolution of viviparity are compatible in our study model.

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Introduction

Maternal effects and environmental conditions during embryonic development have a great influence on an organism's phenotype and can affect fitness in reptiles (Shine and Harlow, 1996; Deeming, 2004). Effects induced by developmental environment have special importance in oviparous species because eggs are exposed to a wide variation of environmental factors, which may have detrimental effects on embryo phenotypes if certain limits are reached (Flatt et al., 2001). Temperature is one of the most relevant environmental factors inducing phenotypic variation in ectothermic vertebrates (Johnston and Bennett, 1996). In squamate reptiles (lizards and snakes), incubation temperature influences incubation time, hatching success and several

hatchling traits (e.g. Birchard, 2004; Deeming, 2004; Booth, 2006). There are, however, interspecific differences in the way that hatchlings are affected by incubation temperature. High incubation temperatures, within the range that produces viable offspring, are detrimental to hatchlings of some lizard species (e.g. *Podarcis muralis*: Van Damme et al., 1992; Braña and Ji, 2000), whereas low incubation temperatures produce "poor-quality" hatchlings in other species (e.g. *Sceloporus virgatus*: Qualls and Andrews, 1999). Furthermore, pregnant females of several lizard species modify their body temperature by increasing or decreasing it in comparison to non-pregnant females (Beuchat, 1986; Braña, 1993; Shine, 2006). The change in preferred temperature during pregnancy could be selected to provide suitable temperatures either for embryonic development or for physiological processes of the female (Beuchat and Ellner, 1987; Beuchat, 1988; Mathies and Andrews, 1997).

In addition to the choice of nest site or the season in which incubation takes place (Shine, 2002; Birchard, 2004), maternal

thermoregulation during the intrauterine phase can importantly affect egg temperature. This could be especially relevant in viviparous lizards or in oviparous species such as *Zootoca vivipara*, in which females retain eggs for an important fraction of development (Braña et al., 1991; this article). *Z. vivipara* (Jacquin, 1797) (Squamata: Lacertidae) is the only lacertid lizard which exhibits intraspecific reproductive bimodality, being viviparous in most populations of its wide Eurasian distribution but oviparous in populations at the southernmost limits of its range (Surget-Groba et al., 2001). Oviparous populations exhibit prolonged egg retention (Braña et al., 1991; Heulin et al., 1991) and likely represent the ancestral stage to current viviparous populations (but see Surget-Groba et al., 2006). While pregnant, females of some populations of *Z. vivipara* select lower body temperatures than do non-pregnant females (see, for viviparous populations, Van Damme et al., 1986; Heulin, 1987; for oviparous populations, Carretero et al., 2005; this article). Therefore, it is likely that temperature plays a relevant role in the embryonic development of this species.

The aim of this study was to assess the influence of reproductive status on maternal thermoregulation and to evaluate the effect of incubation temperature on the duration of embryonic development as well as on some traits of hatchling phenotype that could be relevant for offspring fitness.

There are two outstanding hypotheses on the evolution of viviparity in reptiles. On the one hand, the classical "cold-climate hypothesis" proposes that prolonged uterine egg retention is favoured in cold climates because eggs develop faster at maternal body temperatures than at soil temperatures and retention enhances survivorship in unfavourable climates (Tinkle and Gibbons, 1977). On the other hand, the "maternal manipulation" view proposes that thermal conditions during embryogenesis in utero enhance relevant phenotypic traits of the offspring directly, not through shortening development time (Shine, 1995). Actually, in some instances the cold-climate hypothesis might be considered just a special case of a broader maternal manipulation hypothesis (Webb et al., 2006). However, both views can be conflicting if, for example, temperatures that enhance offspring phenotypes inside the mother's oviduct prolong incubation. Thus, the present study also aims to offer an insight into the compatibility of both hypotheses as well as into the selective forces acting in a critical phase of development in oviparous populations of one of the very few amniote species that exhibit intraspecific reproductive bimodality.

Material and methods

Z. vivipara is a small (adult snout-vent length in our sample: 40–64 mm) ground-dwelling lizard inhabiting a variety of vegetated and wet habitats, such as peat bogs, meadows and humid heathlands. The reproductive phenology of oviparous populations of *Z. vivipara* in the Cantabrian mountain range has been described by Braña (1986): females reproduce once a year and lay clutches of 2–11 eggs; ovulation generally takes place in early June and oviposition generally occurs in July.

Gravid females of *Z. vivipara* were captured by hand from a highland population in Las Señales (León, Northern Spain; elevation: 1650–1750 m a.s.l.) and were transported to the Zoology laboratory at the University of Oviedo. Lizards were housed in terraria which were exposed to the natural photoperiod and in which suspended light bulbs provided the opportunity for behavioural thermoregulation. Dishes of water, food (mealworms and crickets) and an appropriate substrate for laying eggs were also provided. After egg laying, eggs were individually weighed and assigned to one of the incubation treatments. Although we could not get a balanced design to analyse family effects
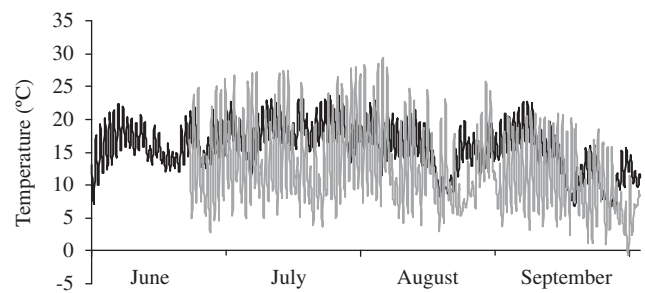

Fig. 1. Two representative series of temperatures registered in potential nests of *Z. vivipara* during the incubation period (one record per hour from June to September). Curves of two different sites: 1650 m a.s.l. in 2006 (black) and 1750 m a.s.l. in 2007 (grey).

(as regards female parent), due to the small clutch size (3–9 eggs per clutch in the used sample), we distributed the eggs as equally as possible among treatments. Anyway, family effects can be considered irrelevant to the overall results because of the high number of females and the multiple paternity within clutches in this species (Laloi et al., 2004).

One egg of each clutch was dissected to ascertain the embryonic stage at oviposition according to the development table by Dufaure and Hubert (1961). The remaining eggs were individually incubated in 50 ml covered plastic containers at one of five constant temperatures: 21, 25, 29, 32 and 34 °C. We kept a constant moisture level at all incubation temperatures (2 g distilled water/1 g vermiculite). In addition, a small number of eggs ($n=4$) were incubated at 17 °C in order to extend the range and improve the fitting of the relationship between incubation time and temperature.

In order to have a general reference about conditions in which external incubation takes place in the field, we carried out continuous recordings of temperature by placing miniature thermal data-loggers (Tidbit v2; Onset Computer Corp., Bourne, MA, USA) in nest locations of clutches of *Z. vivipara* from May to October in 2006 and 2007, including retention (May–June) and external incubation (July–September) periods. Data from two actual and three potential nest locations, i.e. nearby places similar to those where clutches of *Z. vivipara* had been found, were recorded. The two lowest temperatures utilised in our experimental incubations (21 and 25 °C) are in the range of temperatures registered in natural nests of *Z. vivipara* during the external incubation period (Fig. 1). The temperatures 29 and 32 °C are over this range, but they are frequently reached during the early embryonic development inside the mother's body. Finally, 34 °C is an incubation temperature higher than that selected by gravid females in a thermal gradient in the laboratory but is roughly the temperature preferred by males and non-gravid females (see Results).

Modification of body temperature during gestation

According to the model which connects the evolution of viviparity with thermal optimisation in early stages of embryogenesis (Shine, 1995), females modify their body temperature during pregnancy in several lizard species. To check this pattern in our study population, we measured the body temperature of 143 adult lizards active in the field during the reproductive period, using a quick-reading cloacal Schultheis thermometer (Miller & Weber, Inc., Ridgewood, NY, USA). Furthermore, we measured the substrate temperature at the point where the individual was first observed and the air temperature at 10 cm above that point. These data were compared with the temperatures "selected" by the individuals, which behave like heliothermal organisms in a laboratory

thermal gradient where they can regulate their body temperature with a minimum of associated costs (Braña, 1993). Temperature measurements of gravid females were taken during the week before oviposition, so that the embryonic development stage was roughly the same for all gravid females at that time.

A heat source (60 W light bulb) was placed on one side of a terrarium to establish a thermal gradient between the coldest zone (substrate temperature T_s=22.72 $\pm$ 1.70 °C; air temperature T_a=22.84 $\pm$ 0.85 °C) and the warmest one (T_s=37.34 $\pm$ 3.62 °C; T_a=28.36 $\pm$ 1.09 °C). An hour and a half after locating the lizards in the thermal gradient, we measured their body temperatures with a Schultheis quick-reading thermometer. We placed no more than 7 individuals in the gradient to avoid competitive interference for basking places and we took just one measurement of body temperature from each individual.

One-factor analyses of variance were carried out to check for differences between the body temperatures of males, non-gravid females and gravid females in the thermal gradient as well as in the field. Differences in field body temperatures among males, non-gravid females and gravid females were further assessed by analysis of covariance with substrate and air temperatures as covariates. *A posteriori* comparisons were made with Fisher LSD tests.

Effects of incubation temperature on incubation time and hatchling phenotype

Hatchlings were weighed immediately following birth, sexed by eversion of hemipenes in males (Harlow, 1996), and measured using the Image-Pro Plus image processing software. The following measurements were taken: head, abdomen, forelimb, hind limb and tail lengths.

These size measurements were taken to assess how incubation temperature affected each morphological trait separately since each of these traits has an influence on hatchling fitness and most of them are sexually dimorphic. For example, a large abdomen might favour female reproductive success by making larger clutch size or clutch volume possible (Braña, 1996), whereas a large head size favours male reproductive success through enhanced fighting and grasping abilities (Gvoždík and Van Damme, 2003). Besides, head length as well as body size has an influence on bite capacity, an important ecological attribute in adult *Z. vivipara* because of its influence on prey selection (Herrel et al., 2001). Furthermore, males have longer tails than females and Hoffmann and Henle (2006) found that tail length of male *Z. vivipara* is related to their reproductive success, since the longer the tail of males, the higher the number of offspring sired. We also measured the length of digits 2, 3 and 4 (D2, D3 and D4, respectively, numbered from the inner to the outer side) of the right forelimb because sexual dimorphism in digit length ratios has been reported for three species of lizards (Chang et al., 2006; Rubolini et al., 2006). All data were checked for normality (Kolgomorov–Smirnov test) and for homocedasticity (Bartlett's test) prior to further statistical analysis. Some variables (body mass, egg mass, head, abdomen and forelimb lengths) were log-transformed for using parametric tests in the analyses. Incubation time did not meet the above assumptions and did not improve with simple transformations; for this reason, it was analysed by the nonparametric Kruskal–Wallis test. Two-factor analyses of covariance with snout-vent length (SVL) as the covariate were used to determine whether incubation temperature and sex had an influence on body mass, tail, forelimb, hind limb and digit lengths. In the case of head and abdomen lengths we used log-transformed egg mass instead of SVL as the covariate, because these measures are part of SVL. Hatchling mass was not used as the covariate because males are

more robust than females (see Results). The significance level for all tests was set at α=0.05. *A posteriori* comparisons among means were assessed with Fisher LSD tests.

Results

Modification of body temperature during gestation

The mean body temperature preferred by gravid females was lower than that preferred by males and non-gravid females in the thermal gradient (Table 1; ANOVA: $F_{2,216}$=54.449, $P < 0.001$; Fisher LSD test a *posteriori*). Temperatures of lizards active in the field were considerably lower than temperatures selected in the thermal gradient. The mean cloacal temperature of gravid females active in the field was also lower than that of the other two groups (Table 1; ANOVA: $F_{2,140}$=4.977, $P < 0.01$; and ANCOVA: $F_{2,119}$=3.823; $P < 0.05$; Fisher LSD test). In the field, gravid females were found on cooler substrates than the other individuals (Table 1; ANOVA: $F_{2,124}$=3.712; $P < 0.05$; Fisher LSD test). Air temperatures at the lizards' location in the field did not differ among groups (ANOVA: $F_{2,122}$=1.439, $P > 0.05$).

Effects of incubation temperature on incubation time and hatchling phenotype

Eggs dissected at oviposition contained embryos at stages 31–34, according to Dufaure and Hubert's (1961) developmental series. Incubation temperature clearly influenced the duration of embryonic development (Kruskal–Wallis test: H (5, 168)=157.58; $P < 0.001$) according to a negative exponential function (Fig. 2). Incubating eggs at 21 °C reduced incubation time by half (mean $\pm$ s.d.: 40.51 $\pm$ 2.21 d) compared with incubating eggs at 17 °C (86.00 $\pm$ 2.83 d), whereas there were no significant differences in incubation time at temperatures of 32 and 34 °C (12.57 $\pm$ 0.65 and 12.39 $\pm$ 0.50 d, respectively).

There were marked male–female differences in several hatchling traits: males were heavier (relative to SVL) and had longer tails and legs than females, whereas females had a longer abdomen (Table 2). Besides, males had larger digits (D3 and D4) than females (ANCOVAs: $F_{1,121}$=6.854; $P < 0.01$ for D3 and $F_{1,121}$=6.228; $P < 0.05$ for D4). No influence of sex was found on D2 length ($F_{1,121}$=1.623; $P > 0.05$).

Incubation temperature significantly influenced survival and morphology of the survivors. Survival at 34 °C (58%, N=31) was much lower than at the other incubation temperatures (93%, N=156; χ^2 test: χ^2=42.203, degrees of freedom=4, $P < 0.001$). Most dead embryos were full-term ones with deformed heads and unable to hatch. Abdomen and tail lengths were larger in hatchlings from higher incubation temperatures whereas heads were much shorter in hatchlings from the highest incubation

Table 1
Body temperatures (T_b) of adult *Zootoca vivipara* according to their reproductive condition (in thermal gradient and in the field) and substrate and air temperatures in those places where they were in the field (°C, mean $\pm$ s.d.).

	Reproductive condition		
	M	Non-gravid F	Gravid F
T_b gradient	34.25 $\pm$ 0.96 (N=58)	33.94 $\pm$ 1.12 (N=97)	32.33 $\pm$ 1.27 (N=64)
T_b field	29.77 $\pm$ 2.70 (N=39)	29.20 $\pm$ 2.89 (N=41)	27.91 $\pm$ 3.33 (N=63)
T substrate	24.39 $\pm$ 3.60 (N=31)	23.97 $\pm$ 3.57 (N=40)	22.31 $\pm$ 4.14 (N=56)
T air	20.48 $\pm$ 3.15 (N=30)	20.13 $\pm$ 2.77 (N=39)	19.29 $\pm$ 3.83 (N=56)

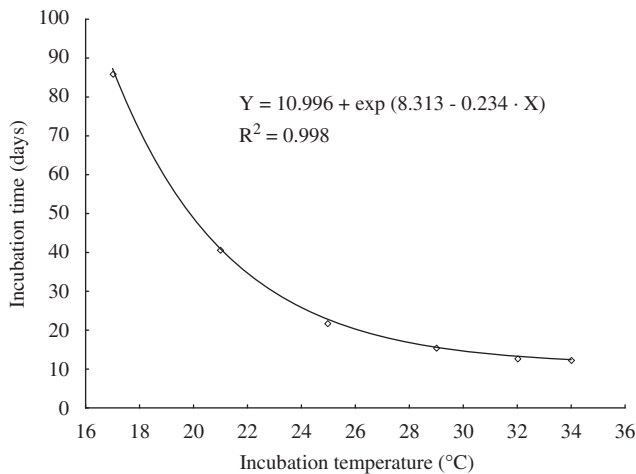Sample sizes corresponding to field and laboratory measurements are in brackets. M=Males; F=Females.

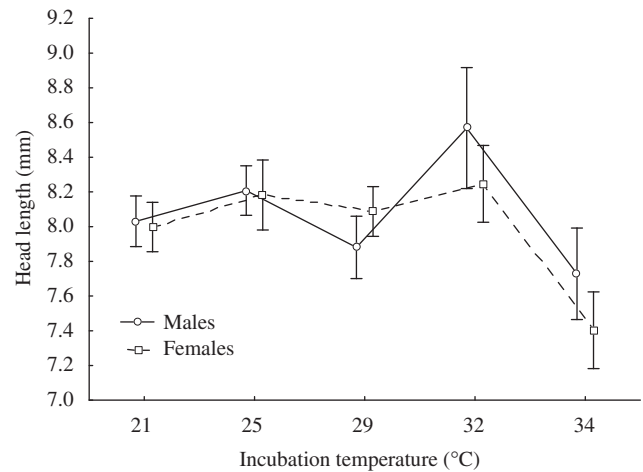Fig. 2. Relationship between incubation temperature (constant) and incubation time (mean) in Z. vivipara.

Fig. 3. Effects of incubation temperature on head length in male and female Z. vivipara (means and 95% confidence intervals).

Table 2
Phenotype of hatchling *Zootoca vivipara* according to sex and incubation temperature (mean ± s.d.).

Hatchling trait		Incubation temperature					Effects		
		21 °C	25 °C	29 °C	32 °C	34 °C	Sex	Temperature	Interaction
Body mass (g)	M	0.219 ± 0.02	0.221 ± 0.01	0.216 ± 0.02	0.234 ± 0.02	0.231 ± 0.03	$F_{1,142}=14.00^{**}$	$F_{4,142}=1.38$	$F_{4,142}=1.13$
	F	0.223 ± 0.01	0.230 ± 0.03	0.226 ± 0.02	0.222 ± 0.02	0.228 ± 0.04	M > F		
Head (mm)	M	8.00 ± 0.29	8.23 ± 0.39	7.88 ± 0.36	8.59 ± 0.37	7.70 ± 0.20	$F_{1,142}=2.23$	$F_{4,149}=11.82^{***}$	$F_{4,142}=2.31$
	F	7.99 ± 0.33	8.22 ± 0.50	8.12 ± 0.44	8.21 ± 0.31	7.38 ± 0.40		(25,32) > (21,29) > 34	
Abdomen (mm)	M	12.15 ± 0.64	12.86 ± 0.98	12.12 ± 0.57	13.15 ± 1.18	$\mathbf{13.76 \pm 1.70}$	$F_{1,142}=90.15^{***}$	$F_{4,142}=14.10^{***}$	$F_{4,142}=2.40$
	F	13.40 ± 0.62	14.12 ± 1.17	13.98 ± 0.90	14.98 ± 0.55	14.30 ± 0.55	F > M	21 < (25,29) < (32,34)	p=0.053
Forelimb (mm)	M	3.83 ± 0.32	4.04 ± 0.52	3.74 ± 0.37	3.99 ± 0.29	3.56 ± 0.52	$F_{1,139}=7.77^{**}$	$F_{4,139}=4.24^{**}$	$F_{4,139}=1.25$
	F	3.73 ± 0.34	3.97 ± 0.38	4.10 ± 0.43	4.18 ± 0.29	3.57 ± 0.38	M > F	(21,34) < (25,29,32)	
Hind limb (mm)	M	4.83 ± 0.55	5.06 ± 0.37	4.90 ± 0.40	5.18 ± 0.32	4.82 ± 0.64	$F_{1,142}=18.94^{***}$	$F_{4,142}=1.85$	$F_{4,142}=0.84$
	F	4.88 ± 0.38	4.86 ± 0.46	4.85 ± 0.46	5.03 ± 0.20	4.60 ± 0.41	M > F		
Tail (mm)	M	23.59 ± 2.70	25.81 ± 1.49	25.60 ± 2.27	26.35 ± 2.39	26.27 ± 2.41	$F_{1,141}=28.50^{***}$	$F_{4,141}=6.74^{***}$	$F_{4,141}=0.46$
	F	23.05 ± 1.03	25.37 ± 1.54	25.02 ± 1.22	25.89 ± 1.50	24.20 ± 2.48	M > F	21 < (25,29,32,34)	

M=male; F=female. $^{**}P < 0.01$; $^{***}P < 0.001$.
F ratios correspond to single effects and between-factor interactions in two-factor ANCOVAs with snout-vent length (for body mass, tail, forelimb and hind limb lengths) or egg mass (for head and abdomen lengths) as the covariate. Parentheses below *F* values for the effects of incubation temperature group together treatments that did not differ as revealed by Fisher LSD tests *A. posteriori*.

temperature (Fig. 3; Table 2). Hatchlings from the lowest and the highest incubation temperatures had shorter forelimbs.

There was a nearly significant interaction between sex and temperature treatments regarding abdomen length (P=0.053; see Table 2). Sexual dimorphism in abdomen length was not significant in hatchlings from the highest incubation temperature (34 °C), while it was significantly different in hatchlings from the other temperatures.

Discussion

Results of the present study indicate that incubation temperature influences development time, survival and a number of phenotypic traits of hatchling *Z. vivipara*. In several respects, and especially regarding survival rates during the incubation time, high temperatures produced negative effects on development. Experimental incubations in the lacertid *P. muralis* have also shown that high incubation temperatures produce detrimental effects on hatchling morphology and fitness (Ji and Braña, 1999; Braña and Ji, 2000), even if they are applied only during the earliest phase of the extrauterine incubation (Braña and Ji, 2007).

P. muralis lays eggs containing embryos at developmental stages 25–29 (according to the development table by Dufaure and Hubert, 1961), and therefore, the early extrauterine incubation phase in this species corresponds to the retention phase in *Z. vivipara*, a species in which embryos are at more advanced stages at oviposition. For this reason, although effects of thermal conditions on egg development and hatchling traits were tested during external incubation in the present study, we assume that these effects also apply to the intrauterine incubation period and thus our results may also help to explain modifications of basking behaviour of females during egg retention. Gravid females of *Z. vivipara* selected lower temperatures in the laboratory thermal gradient than did males and non-gravid females, just like other closely related lacertid lizard species (*P. muralis*: Braña, 1993) and other viviparous (Van Damme et al., 1986; Heulin, 1987) and oviparous (Carretero et al., 2005) populations of the same species. The fact that there were no costs of predation or food supply along the laboratory thermal gradient supports the idea that low temperatures of gravid females were not a consequence of restricted opportunities of thermoregulating because of their limited mobility (Le Galliard et al., 2003). The pattern might be related to the fact that the optimal temperature for embryonic

development is lower than the optimal temperature for most physiological functions of females and, therefore, gravid females would select lower temperatures to optimise reproductive success (Braña, 1993).

Field body temperatures were considerably lower than temperatures selected in the laboratory thermal gradient, and this may indicate that the thermal environment of our highland study population makes accurate thermoregulation difficult. Field body temperatures of pregnant females were also lower than those of other adult animals and gravid females occupied places with lower substrate temperatures than males and non-gravid females. The fact that pregnant females occupy places with lower substrate temperatures may indicate that they are active even at environmental temperatures below the normal activity range but allow some increase of body temperatures via behavioural thermoregulation, because this accelerates embryonic development in utero.

Shine (2004) proposed that thermal variation might be the main feature optimised by maternal retention, and some other studies support this conclusion (Webb et al., 2006). Our data apparently support this possibility, as temperatures registered in the lizard nests were much more variable (mean ± s.d. temperature in actual nests: 16.74 ± 2.68; 14.98 ± 3.44 °C) than those maintained by pregnant females when active. However, activity and thermoregulatory time make up only approximately one third of the day, and pregnant females probably spend the remaining time at or near soil temperature; this would imply, in fact, higher thermal diel variation, as the temperature during activity should be much higher than during inactivity. On the other hand, there is no evidence in our data of significant differences in the thermoregulatory precision of pregnant vs. non-pregnant females either in the field or in the laboratory gradient, as might be expected if extended egg retention had evolved to provide low thermal variation to developing embryos. In fact, pregnant females had lower mean temperatures but also lower minimal activity temperatures as they were active on cooler substrates than non-pregnant females.

Our data support findings of previous studies in lacertid lizards and show that incubation time decreases with increasing temperature (Braña and Ji, 2007). Low incubation temperatures within the range analysed in this study could affect hatchling survival because the longer the hatching is delayed the longer the eggs are exposed to adverse biotic or abiotic factors in the nest. Moreover, considering that in our study population egg laying mostly started in late June, hatchlings from eggs incubated at low temperatures would have a shorter growth period before the onset of winter dormancy. Delayed hatching would imply overwintering with lower fat reserves (Elphick and Shine, 1998; Qualls and Andrews, 1999) and, through reduced growth prior to winter, may also delay reproductive maturity (Warner and Shine, 2007), likely affecting life-time fitness.

Two main hypotheses have been proposed to explain the evolution of viviparity through progressive egg retention: (i) the "cold-climate hypothesis" posits that egg retention has evolved to accelerate development in cold environments where low nest temperatures would retard developmental progress (Packard et al., 1977; Shine, 1985); (ii) the "maternal manipulation hypothesis" (Shine, 1995; Webb et al., 2006) posits that females retain eggs to maintain suitable conditions for embryonic development. One critical assumption of the maternal manipulation hypothesis is that the phenotypic changes induced by maternal thermoregulation should enhance offspring characteristics, thereby influencing fitness. Results of our experimental incubations indicate that incubation at 34 °C (near the temperature selected by non-gravid females) negatively influences hatchling survival: about 42% of eggs incubated at this temperature

produced unviable offspring (as reported for *P. muralis* by Ji and Braña, 1999). Additionally, survivors from this incubation temperature had smaller heads and fore limbs, which could imply a lower fitness because (i) head size has an influence on bite capacity (Herrel et al., 2001) and favours male reproductive success (Gvoždík and Van Damme, 2003); and (ii) forelimb length may influence locomotor performance (Braña, 2003), which plays a relevant role in the ability of escaping from predators, obtaining food, and in interspecific interaction. Assuming that high temperatures would have the same effect on oviductal eggs as on oviposited eggs, our results indicate that the temperature selected by males and non-gravid females would be detrimental to survival and to the phenotypes of hatchlings, and thus may explain why females select lower body temperatures when gravid. In addition, this provides a plausible evolutionary scenario for the increased occurrence of egg retention since it extends the time the embryos are exposed to suitable temperatures (Braña and Ji, 2007). Therefore, our results are consistent with expectations under the "maternal manipulation hypothesis", as pregnant females modify their body temperature and avoid keeping eggs at temperatures that negatively influence hatchling characteristics. On the other hand, the fact that gravid lizards "select" lower body temperatures than similarly sized males and non-pregnant females would seemingly contradict expectations under the "cold-climate hypothesis", because the decrease in temperature during pregnancy would retard embryonic development. However, the shift of thermal preference of pregnant females is not incompatible with the acceleration of development proposed by the cold-climate model. Because of the nonlinearity of the relationship between temperature and incubation time, the temperature selected by females during the time of egg retention would result in only a very short delay, if any, of hatching. If eggs are kept at 34 °C (close to the temperature selected by non-gravid females) during the external incubation period, there is only a slight decrease in incubation time with respect to those incubated at 32 °C (close to the temperature selected by gravid females). If a similar rate of thermal dependence occurs during the phase of intrauterine development, egg retention would considerably shorten incubation time, as the temperature selected by pregnant females, although lower than the one selected by non-pregnant females, is much higher than average temperatures recorded in natural nests of *Z. vivipara*.

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